Evaluation of Factors which Stabilize Transgene Expression in Plants

Taniya Dhillon, Joseph M. Chiera and John J. Finer

Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center/The Ohio State University, Wooster, OH 44691

ABSTRACT

Regulation of gene expression in eukaryotes is a complex phenomenon. Over-expression of a gene may result in its "silencing". This phenomenon called RNA silencing is an anti-viral defense mechanism in plants. Viruses encode certain proteins called suppressors of silencing, which suppress the RNA silencing defense mechanisms of plants. For our research, different plant viral suppressors were evaluated for their ability to stabilize expression of the Green Fluorescent Protein (GFP) using a unique transient expression assay. DNA was introduced into lima bean cotyledons via particle bombardment and automated image collection and analysis were used for quantification and tracking of GFP expression. Introduction of the *gfp* gene under regulatory control of the constitutive 35S promoter resulted in GFP expression lasting only for about 72 hrs post bombardment. However, introduction of DNAs with the *gfp* gene fused to the different viral suppressors resulted in significant extensions of GFP expression. HC-Pro (Tobacco Etch Virus) and P19 (Tomato Bushy Stunt Virus) were among the strongest stabilizers of transgene expression, extending GFP expression up to 120 hrs. Although it is still unclear if these viral suppressors are indeed functioning as silencing suppressors in this system, there is a clear extension and stabilization of transgene expression. Evaluation of the stabilizing effects of these DNA fragments using the lima bean cotyledon model, together with automated image collection and analysis, has provided a consistent mechanism for comparing the relative "strength" of the gene expression stabilizers. These stabilizers could potentially provide more consistent transgene expression in plants and other organisms.

INTRODUCTION

Gene transfer can be used to study gene expression or produce transgenic organisms with desired novel characteristics. However, transgene introduction may sometimes lead to over-expression of the gene resulting in gene silencing. This phenomenon, called RNA silencing (1), nullifies the efforts put towards production of transgenics. Plants also employ RNA silencing as an anti-viral defense mechanism. In retort, viral genes encode proteins that suppress the RNA silencing defense mechanisms of plants (5). These viral proteins, called suppressors of silencing, could potentially be exploited to enhance transgene expression in plants and other organisms. Our research aims to evaluate the effects of some of the suppressors on transgene expression using a unique transient expression assay. For this work, DNA constructs of the suppressors fused to the *green fluorescent protein* (*gfp*) gene (2) were introduced into lima bean cotyledons (Fig. 1) and GFP expression was tracked over time. The use of the lima bean model system, along with a consistent approach towards quantification and analysis of the results has allowed us to compare, in a novel way, a number of different viral suppressors.

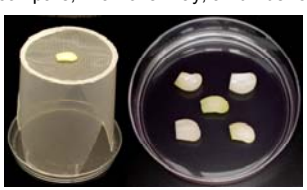

Figure 1. Lima bean cotyledon placed flat side up on the holey baffle for bombardment (left). Five cotyledons aligned in a Petri dish for automated image collection (right).

MATERIALS AND METHODS

The suppressors chosen for the study include HC-Pro (Tobacco Etch Virus), P19 (Tomato Bushy Stunt Virus), AL2 (Tomato Golden Mosaic Virus), P21 (Beet Yellows Virus), γb (Barley Stripe Mosaic Virus) and Replicase (Tobacco Mosaic Virus). Vector constructions of the *gfp* gene fused to the different viral suppressors, under the regulatory control of the constitutive 35S promoter, were synthesized. Suppressor vectors without GFP were also constructed in the case of HC-Pro, P19 and AL2 for co-introduction with the *gfp* plasmid.

Fresh lima beans, harvested from the plants in the greenhouse, were surface-sterilized and germinated on sterile, moistened paper towels. Lima bean cotyledons were excised and the different vector constructions were introduced into the cotyledon cells via particle bombardment (4). GFP expression was tracked over time using a robotics system (Fig. 2) programmed for automated image collection at an hourly interval. Analysis of the images (Fig. 3) was done using Wright Cell Imaging Facility (WCIF) Image J Program for the quantification of GFP expression in terms of GFP intensity, number of foci expressing GFP and average size of the foci.

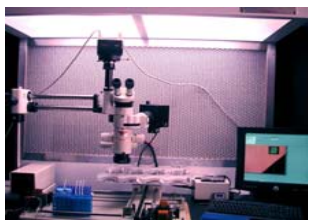

Figure 2. The robotics system for tracking of GFP expression, provided with two dimensional plexiglass platform, fluorescence dissecting microscope and digital camera, under computer control placed in a laminar flow hood (3).

RESULTS

Introduction of *gfp* gene under the regulatory control of the constitutive 35S promoter in lima beans results in GFP expression enduring for only about 72 hrs post-introduction. However, fusion of *gfp* gene to the different suppressors extended GFP expression to 120 hrs post-introduction. Co-introduction of *gfp* and viral factors (HC-Pro and P19) on separate plasmids, however, did not result in considerable stabilization of GFP expression (Fig. 4).

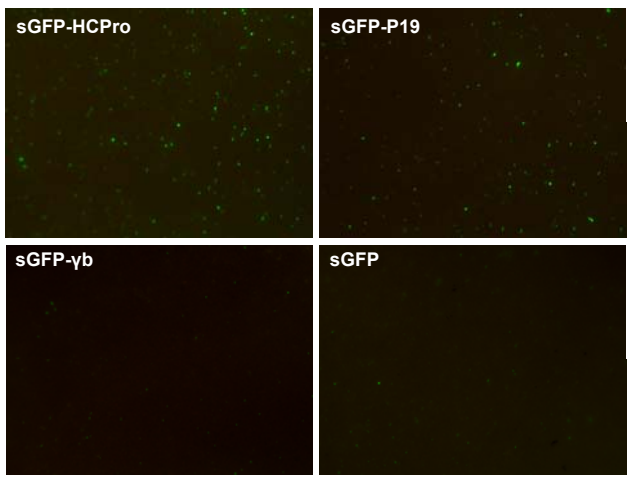

Figure 3. Comparison of the images collected by the robot (at 100 hrs post-introduction) showing the effect of HC-Pro, P19 and γb on stabilization of GFP expression as opposed to the presence of *gfp* gene alone.

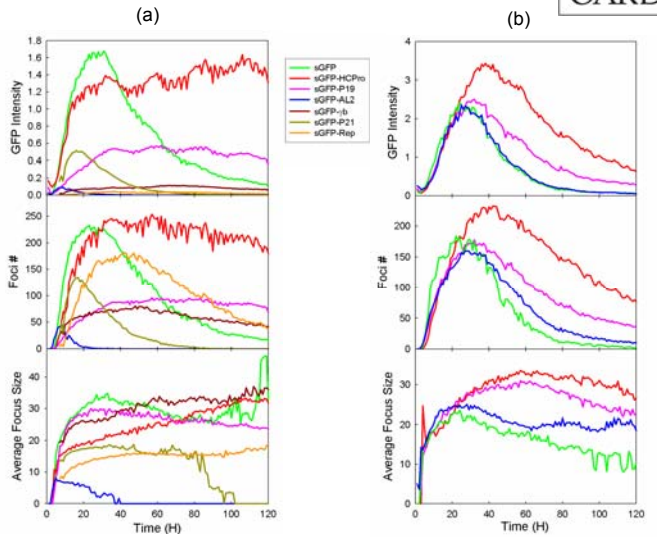

Figure 4. Quantification of GFP expression in lima bean cotyledons of (a) sGFP-suppressor fusion introductions and (b) co-introductions using Image J program.

CONCLUSIONS AND DISCUSSION

Transient transgene expression was extended in the presence of viral suppressors of silencing. Though it is not clear if these viral sequences are working as silencing suppressors or some other type of stabilizer/enhancer in our system, it is quite evident that these DNAs stabilized transient gene expression.

- HC-Pro was the strongest among all the viral factors evaluated for transient transgene stabilization, followed by P19.
- AL2 and P21 did not extend GFP expression in the lima bean model system; they reduced GFP intensity and number of foci.
- The nuclear localization signal of AL2 apparently targeted GFP to the nucleus only when the *gfp* gene and AL2 were introduced *in-cis.*
- γb stabilized GFP expression up to 120 hrs, although the intensity of expression was extremely low.
- The different vector constructs will be stably introduced into soybean (*Glycine max* L. Merill) embryogenic tissue to evaluate their effect on transgene expression in stably-transformed tissue.

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